MYCORRHIZA INOCULATION IN FORESTRY PRACTICE

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SUMMARY

Microbiological factors in mycorrhiza inoculation of trees are discussed with emphasis on Australian research results. These include natural occurrence of mycorrhiza fungi, differences in efficiency of mycorrhizas and establishment of inoculum.

It is now beyond doubt that under certain conditions inoculation of tree species with mycorrhiza fungi increases tree growth. Responses to inoculation have been shown with such diverse trees as *Pinus* spp. (e.g. Kessell 1923, Young 1936, Hatch 1937), *Eucalyptus* spp. (e.g. Pryor 1956), Norway spruce, birch (Levisohn 1957), oak (Shemekhanova 1961) and others. Although studies on microbiological, ecological and physiological aspects of mycorrhiza relations of *Pinus radiata* D. Don. are in progress in South Australia this article discusses some of the microbiological factors only, involved in inoculation and tree response assuming that environmental conditions are suitable for a response. Almost by tradition this response has been attributed to increased nutrient uptake from soils low or unbalanced in nutrients and, indeed, increased nutrient uptake by mycorrhizal plants is well established (Harley 1959). However, it should never be forgotten that the mycorrhiza is quite another type of root and may differ from the uninfected root in many properties; e.g. Zak (1964) has advanced that mycorrhizas may be more resistant to the plant pathogen *Phytophthora cinnamommi* than unsuberized, uninfected roots.

Basically the microbiological questions of response in a particular soil revolve around two considerations:

(i) the natural occurrence of mycorrhiza fungi for the particular tree species

and

(ii) differences between fungi in their efficiency when associated with the tree. In practice other questions also are involved and these include successful introduction of fungi into existing soils in competition with the native microflora, and the survival of the introduced fungus in effective quantity from season to season.

Natural Occurrence of Mycorrhiza Fungi

In parts of Europe mycorrhiza fungi for pine species are ubiquitous (Bjorkman 1961) but this is not so in many other parts of the world (Kelley 1950). In Australia the earliest record of response to inoculation of *P. radiata* was by Kessell (1923) in Western Australia, in soils where mycorrhiza fungi for this species were absent. Young (1936) observed similar responses by *P. caribaea* and *P. patula* in Queensland, and more recently

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Pryor (1958) has reiterated the need for introduction of suitable mycorrhiza fungi when introducing exotics.

In South Australia Samuel (1926) recorded sporadic natural mycorrhiza formation on *P. radiata* in recently cleared soils. Natural occurrence of fungi for this species was studied more intensively by Bowen (1963) by sampling 78 sites in South Australia. Spread apparently occurred for one to two miles from forests, but of the 51 sites well removed from forests 26 contained mycorrhiza fungi. A more detailed analysis with regard to existing vegetation (Table 1), showing occurrence of mycorrhiza fungi for *P. radiata* in eucalypt stands, was meaningful with respect to Samuel's (1926) observation in that the areas he referred to almost certainly carried *Eucalyptus* species naturally. Recently Trappe (1964) reported the occurrence of the mycorrhiza fungus *Cenococcum graniforme* on *E. gigantea* as well as on several other trees including *P. radiata,* while Mikola (1948) showed that this fungus occurred in soils with no known host. Bowen and Theodorou (unpubl.) have recently shown its ability to colonize grass roots. Growth of mycorrhiza fungi in the rhizosphere of other plants need not mean that they are capable of forming mycorrhizas with these plants, but has ecological importance in providing a reservoir of the fungus in the absence of a mycorrhiza-forming host. This field of study has been little explored so far. The general lack of mycorrhiza fungi for trees in grassed areas shown in Table 1 confirms similar reports (Wilde 1954, Goss 1960, Curtis and Cottam 1950) which postulated the production by grasses of toxins towards the mycorrhiza fungi.

TABLE 1

Occurrence of mycorrhizas on *P. radiata* in relation to vegetation (from Bowen, 1963).

Vegetation	Number of soils containing no mycorrhiza	Number of soils containing mycorrhiza
Grass and treeless	6	2
Grass with scattered *Eucalyptus*	8	6
Predominantly *Eucalyptus*	10	20

Although several South Australian soils contained one or more mycorrhiza fungi for *P. radiata,* the percentage of infected roots was low in many cases, probably reflecting a low population. In these cases an increase in infection and tree response following inoculation could well occur as the natural spread of mycorrhiza fungi through soil can be slow (Tserling 1960).

Differences in Efficiency Between Fungi

Increases from inoculation of soils already carrying mycorrhiza fungi might be expected if more efficient fungi were introduced successfully. This involves not only the question of differences in plant response in a particular environment to different fungi, but also the range of fungi which may form mycorrhizas on that plant.

Because of the difficulties involved in the isolation of mycorrhiza fungi from mycorrhizas there has been a tendency to study only those fungi producing fruiting bodies. Furthermore, forestry workers have largely restricted their thinking to these fungi, and have been prone to draw conclusions about the ecology of particular mycorrhiza fungi from the occurrence of the fruiting body. Such thinking is basically unsound, and could lead to wrong conclusions. The existence of a fruiting body tells little more than that conditions are correct for fruiting. It does not tell whether the particular mycorrhiza fungus is the major (or even a major) symbiont in the stand. More recently the author, and Bryan and Zak (1963) in the U.S.A., in isolating from mycorrhizas have shown several mycorrhiza fungi to be present, from many of which it is difficult to produce fruiting bodies. Zak and Marx (1964) isolated 22 mycorrhiza fungi from *P. elliottii* and occasionally two or three different fungi from the same mycorrhiza. Isolations from mycorrhizas are not always successful due possibly to absence of growth factors from the host in laboratory media and this has led the author into an examination of root exudates produced by *P. radiata*. There are several, including some 16 amino acids (Bowen, in preparation).

Attempts have been made to classify different mycorrhiza types on morphology of the mycorrhizas such as the degree of branching and the colour, appearance and structure of the fungus sheath of the mycorrhiza and Dominik (1956) has described 12 major subtypes and 48 "genera". While this is certainly useful, in very few cases has a particular type of mycorrhiza been proved to be caused only by a particular fungus. Young (1937) noted that mycorrhizas produced by *Rhizopogon luteolus* on *P. taeda* were the same in appearance as those produced by *Boletus granulatus*. Furthermore, mycorrhiza appearance changed within a few days of formation. Redmond (1955) has shown a marked effect of small temperature changes on thickness of the fungus sheath of mycorrhizas of birch in Canada. The uncertainties of whether the one fungus may or may not give different mycorrhiza characters, depending on the environment, of whether several fungi can produce the same apparent type of mycorrhiza, and of recognition of different fungi by mycelial characters in laboratory media, call for high percentage success in isolation and more critical criteria for identification of the symbiotic fungi in ecological studies of mycorrhizas.

What evidence is there that any one mycorrhiza fungus is more efficient than another? To some extent this question has built into it the allocation of a particular function to the mycorrhiza, but there is no real guarantee that increased efficiency in one function means increased efficiency in all functions. There is surprisingly little data on differential response to mycorrhiza fungi but such differences have been demonstrated by Young (1940) with *Pinus caribaea,* Moser (1956) with larch, and Levisohn (1957) using Norway spruce and birch. The reasons for these differences have not been examined. Bowen (1962) by using P^{32} tracers was able to show large differences in uptake of phosphate by excised and intact *P. radiata* mycorrhizas of different types in short term laboratory experiments (e.g. Table 2), and these could be ascribed to differences in the amount of fungus sheath (vide also Harley and McCready 1950), the size of the forked root following infection, and differences in uptake at the cellular level.

In these short term laboratory studies the poorest performer was the type of mycorrhiza which occurred naturally most frequently in South Australian soils. The physiological bases of these differences are being studied more intensively, while differences in plant response in the glasshouse are being examined further.

It is not difficult to envisage differences between mycorrhiza fungi in functions other than nutrient uptake efficiency, e.g. Lindeberg (1948) found that *Boletus subtomentosus,* a mycorrhiza former with *Pinus montana,* was somewhat atypical in that it could cause a rapid breakdown of leaf litter. Different mycorrhiza fungi probably have different abilities to "short-circuit" the normal cycle of decomposition and nitrification usually considered necessary for growth of higher plants.

TABLE 2

The uptake of phosphorus by excised mycorrhizas of *P. radiata* (from Bowen, 1962)

Collection Site	Type	Phosphorus Uptake* 10^{-8} (~~10^{8}~~ moles P.)	
		Average per forked root	Average per mg
Kuitpo Forest	(i)	0.70	7.5
	(ii)	3.5	15.5
	Uninfected	(0.4)	3.5
Belair Nursery	(iii)	2.5	15.0
	(iv)	1.5	5.5
	Uninfected	(0.7)	5.5

10^{-3}

* Uptake from 1 x ~~10^{3}~~ molar dihydrogen potassium phosphate for 2 hours at 25°C, pH buffered at 5.5.

It is not too early then to consider that there are potential differences in mycorrhiza characters and that by application of the "correct" fungi we might change the normal characteristics of the root. This leads to the last question to be raised here, namely, the establishment of a selected fungus in practice.

The Establishment of a Selected Fungus in Practice

There have been several reports of difficulty in introduction of pure cultures of mycorrhiza fungi in the mycelial stage into non-sterile soils (Bjorkman 1961). Tserling (1960) found that introduction of mycorrhiza fungi into larch nurseries resulted, not in complete infection, but in foci of infected trees for later spread. This spread however may be very slow. Rayner and Neilson-Jones (1944) and Levinson (1957) have pointed out a sensitivity of mycorrhiza fungi to competitors and antagonists while Melin (1946) demonstrated stimulative and inhibitory substances to mycorrhiza fungi in extracts of leaf litter and humus. Despite the difficulties of superimposing selected mycorrhiza fungi on an existing microflora, this is obviously necessary, not only to avoid the vagueness of older litter spreading techniques but also to reduce the risk of concomitant introduction of pathogens. Robertson (1954) has demonstrated that spores can serve as inoculum, but this approach is limited to those fungi which readily produce fruiting bodies. Theodorou (personal communication) has consistently successfully introduced pure cultures of *Rhizopogon luteolus* and *Boletus granulatus* for *P. radiata* mycorrhiza into Belair nursery soil partially sterilized with methyl bromide, and Moser (1958, 1959) has successfully introduced mycorrhiza fungi into larch and spruce nurseries in Austria with fungi grown on sterilized peat or on needle litter with nutrients added.

One must, of course, look further than the first season and must select fungi which will survive the hot dry southern Australian summer in sufficient quantity to readily re-establish in the following season. Unfortunately, there is very little known about the behaviour of mycorrhiza fungi in soil.

Differences occur in ability of mycorrhiza fungi to colonize the roots of *P. radiata* under aseptic conditions (Bowen, unpublished), and numerous authors (Harley 1959) have demonstrated fungus differences in growth response in laboratory media to environmental conditions, such as temperature and pH. The interplay of environment on survival and growth of the fungus in both the soil and rhizosphere, and on infection processes per se, finds expression in mycorrhiza formation under different conditions. Thus, Theodorou (in preparation) recorded 33 percent of *P. radiata* roots to be infected in a Mt. Burr soil at pH 6.2, 23 percent at pH 4.5 and only 13 percent at pH 8.0.

Furthermore, at pH 8.0 most mycorrhizas were formed by a *Cenococcum graniforme* type. Similarly Worley and Hacskaylo (1959) showed that soil moisture regime had a great effect upon different mycorrhiza types; a white fungus was the predominant mycorrhiza former on *P. virginiana* under relatively high moisture levels (frequent watering) but was completely replaced by a black fungus at a lower moisture level.

Although some progress has been made, there is a great deal yet to be learnt about growth and survival of mycorrhiza fungi in soil and rhizosphere in relation to the physical, chemical and biotic environment.

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